GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION
SPONSORED PROJECT INITIATION

Date: August 29, 1980

Project Title: COMPARATIVE RAMAN STUDIES OF HUMAN AND ANIMAL LENSES

Project No: G-33-A05

Project Director: DR. NAI-TENG YU

Sponsor: DHEW/PHS/NIH - NATIONAL EYE INSTITUTE; BETHESDA, MD 20014

Agreement Period: From May 1, 1980 Until April 30, 1981 (05 YEAR)

Type Agreement: GRANT NO. 5 RO1 EY01746-05

Amount: $48,429 PHS Funds (G-33-A05)
2,550 GIT Contribution (G-33-318)
$50,979 TOTAL

Reports Required: ANNUAL PROGRESS REPORTS WITH CONTINUATION APPLICATIONS; TERMINAL PROGRESS REPORT UPON GRANT EXPIRATION

Sponsor Contact Person (s):

Technical Matters

GEORGE M. STEINBERG, Ph.D.
EXTRAMURAL PROGRAM DIRECTOR
CATARACT PROGRAM
NATIONAL EYE INSTITUTE
BETHESDA, MD 20014

Contractual Matters
(thru OCA)

GAYE LYNCH
GRANTS MANAGEMENT CONTACT
EXTRAMURAL SERVICES BRANCH
NATIONAL EYE INSTITUTE
BETHESDA, MD 20014
PHONE: (301) 496-5884

NOTE: FOLLOW-ON PROJECT TO G-33-A04 (04 YEAR)

Defense Priority Rating: N/A

Assigned to: CHEMISTRY (School/Laboratory)

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GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

SPONSORED PROJECT TERMINATION

Date: August 20, 1981

Project Title: Comparative Raman Studies of Human and Animal Lenses

Project No: G-33-A05

Project Director: Dr. Nai-Teng Yu

Sponsor: DHEW/PHS/NIH - National Eye Institute; Bethesda, Md 20014

Effective Termination Date: 4/30/81

Clearance of Accounting Charges: -----

Grant/Contract Closeout Actions Remaining:

_ Final Invoice and Closing Documents
_ Final Fiscal Report
x Final Report of Inventions
_ Govt. Property Inventory & Related Certificate
_ Classified Material Certificate
x Other Annual Report of Expenditures due by 7/31/81

NOTE: Follow-on project (06 year) is G-33-A06.

Assigned to: Chemistry (School/Laboratory)

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G-33-A05

SECTION IV

APPLICANT: REPEAT GRANT NUMBER SHOWN ON PAGE 1 →	GRANT NUMBER	
SECTION IV—SUMMARY PROGRESS REPORT	5 R01 EY 01746-05	
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR (Last, First, Initial) Yu, Nai-Teng	PERIOD COVERED BY THIS REPORT	
	FROM	THROUGH
NAME OF ORGANIZATION Georgia Institute of Technology	05/01/80	02/10/81

TITLE (Repeat title shown in Item 1 on first page)
Comparative Raman Studies of Human and Animal Lenses

1. List publications: (a) published and not previously reported; (b) in press. Provide five reprints if not previously submitted.
2. List all additions and deletions in professional personnel and any changes in effort.
3. Progress Report. (See Instructions)

1. (b) John F. R. Kuck, Nai-Teng Yu and Carl C. Askren "Total Sulfhydrul by Raman Spectroscopy in the Intact Lens of Several Species: Vibrations in the Nucleus and Along the Optical Axis During Aging" Exp. Eye Res. (in press).

2. Dr. M. Bando of Tokai University (Dept. of Ophthalmology) will join my group in May 1981 for at least two years. His work will involve chromatography and electrophoresis of lens constituents, including membrane proteins. The isolated materials will be subject to Raman and fluorescence studies.

3. Summary Progress Report

(1) Objectives:

a. The overall objectives of the project are: (i) to establish the structural similarities and differences between animal models and human lens; (ii) to identify the nature of lens protein alterations in relation to aging, evolution and cataract formation; (iii) to make careful measurements of sulfhydryl and "fluorophors" profiles in intact human and animal lenses, and determine their age-related changes; (iv) to interpret the dramatic difference found in lens aging between human and rat (or mouse) in terms of the three-dimensional structure of γ-crystallin; (v) to determine if the oxidation of protein sulfhydryl to form disulfide cross-links is involved in human senile cataract.

b. Goals set for the current year: (i) to complete the -SH profile measurements on rabbit, human and chicken; (ii) to obtain the wavelength dependence of fluorescence spectra in aging human lenses; (iii) to apply the new multichannel difference Raman technique developed in this laboratory for revealing protein secondary and tertiary structural changes associated with lens aging process.

(2) Main Scientific Findings and Their Significance:

a. Fluorescence spectra of pigmented human (age 23-97, normal and brunescence) and animal (chipmunk, ~1 year) lenses have been obtained with laser excitation at 406.7, 413.1, 454.5, 457.9, 465.8, 476.5, 488.0, 496.5, 501.7, 514.5, 520.8, 528.7, 530.9, 568.2, 600.0, 610.0, 620.0, 630.0, 647.1 and 676.4 nm. All the lenses examined exhibited strong and similar fluorescence when the excitation wavelengths are shorter than 460 nm. However, with excitation at 568.2 nm or longer the yellow pigments in chipmunk lens show dramatically different emission properties from those in the older and brunsecent human lenses. The near-red and ref fluorophors which were demonstrated to be characteristically elevated in human brunescence are completely absent in chipmunk lens. Upon excitation at 676.4 nm we observed a new fluorophor

in brunescence human lenses (age 35-94) with its emission maximum at ~707 nm (far-red) which is so far the longest emission wavelength detected in ocular lenses. The levels of near-red, red and far-red fluorophors appear to parallel the accumulation of visible pigments in brunescence. We suggest the possibility that emission at 633, 672 or 707 nm may be employed as an indicator for metabolic aberrations in the lens and thus serve as a probe for the in vivo monitoring of such aberrations (see Figs. 1-3).

(b) The sulfhydryl concentration in the central nucleus of rat and mouse lenses falls precipitiously with age. However, in the lenses of man and water buffalo the sulfhydryl decreases at a much slower rate with age. This difference between the two groups appear to be correlated with the derivation of albuminoid: in the rodents it is chielfy γ-crystallin which give rise to albuminoid while in human and bovine lenses albuminoid is related to α-crystallin. The sulfhydryl concentration profiles along the visual axis of human, rabbit and chicken lenses of several ages show that these species have profiles unlike those of rat and mouse lenses; the rabbit lens is more like the human lens while the chicken lens is in a class by itself due to the predominance of δ-crystallin in the nucleus and the consequent extremely low concentration of sulfhydryl.

(c) Studies on intact lenses (mouse and rat) by Raman spectroscopy have demonstrated that γ-crystallin in the central nucleus undergo almost complete oxidation of sulfhydryls to disulfides within 50% life expectancy. It is of interest to examine the effects of disulfide formation on secondary and tertiary structures of γ-crystallins in the intact state. Raman "amide III" band near 1240 cm^{-1} reveals secondary structure, while Tyr "doublet" near 840 cm^{-1} and a Trp ring mode at 878 cm^{-1} reveal tertiary structure. Difference Raman spectra between young (~1 month) and old (8-12 months) lenses display 3 positive bands between 800-890 cm^{-1} but complete cancellation of "amide III" signals, indicating significant changes in tertiary structures but not in secondary structure.

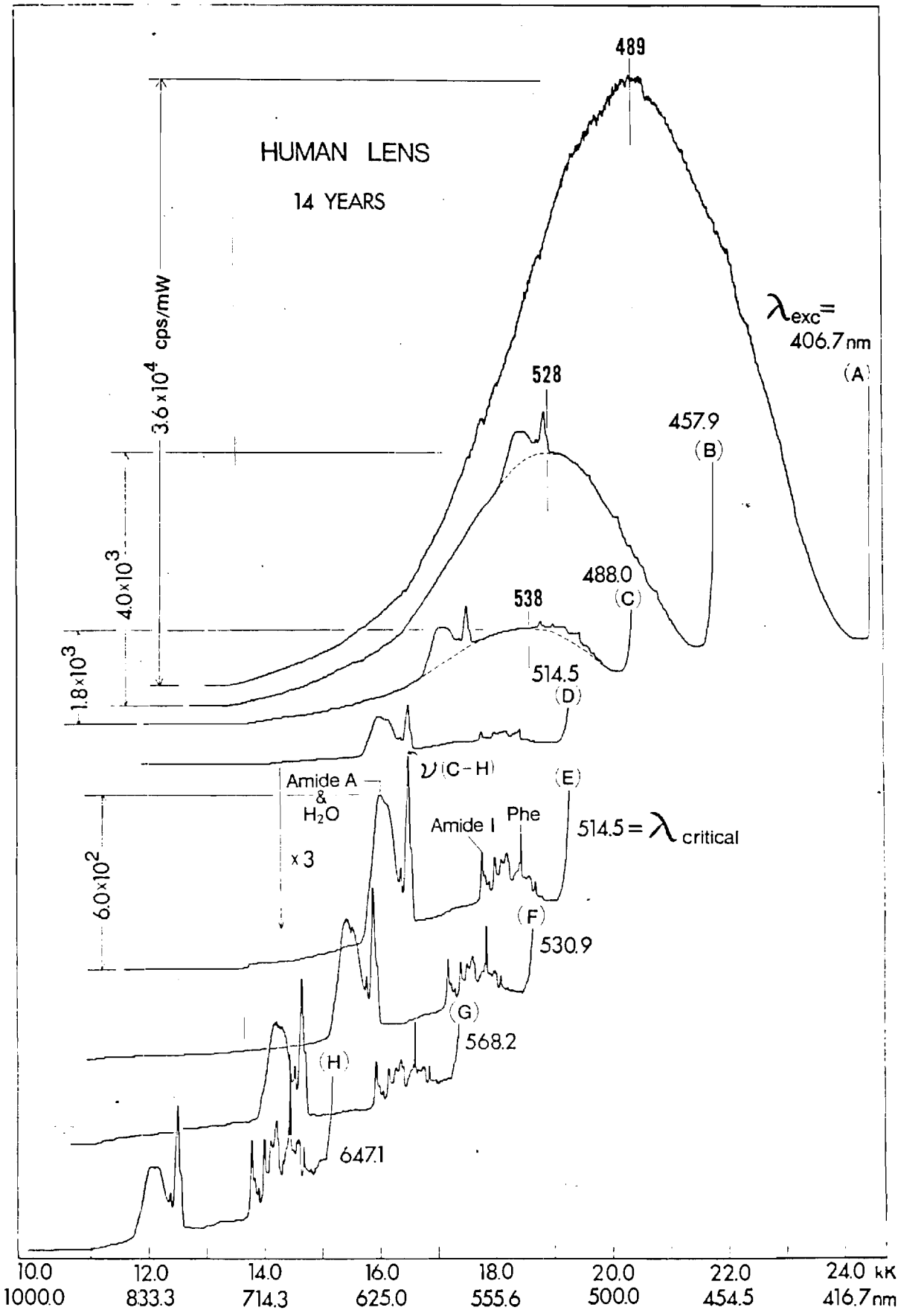
The absence of changes in secondary structure implies that all the S-H groups must be clustered together and/or so arranged on the molecular surface that intra- and inter-disulfide crosslinks are readily formed during normal aging without involving protein unfolding. The factors responsible for the marked difference in age-related behavior between mouse/rat and bovine/human γ-crystallins are under investigation.

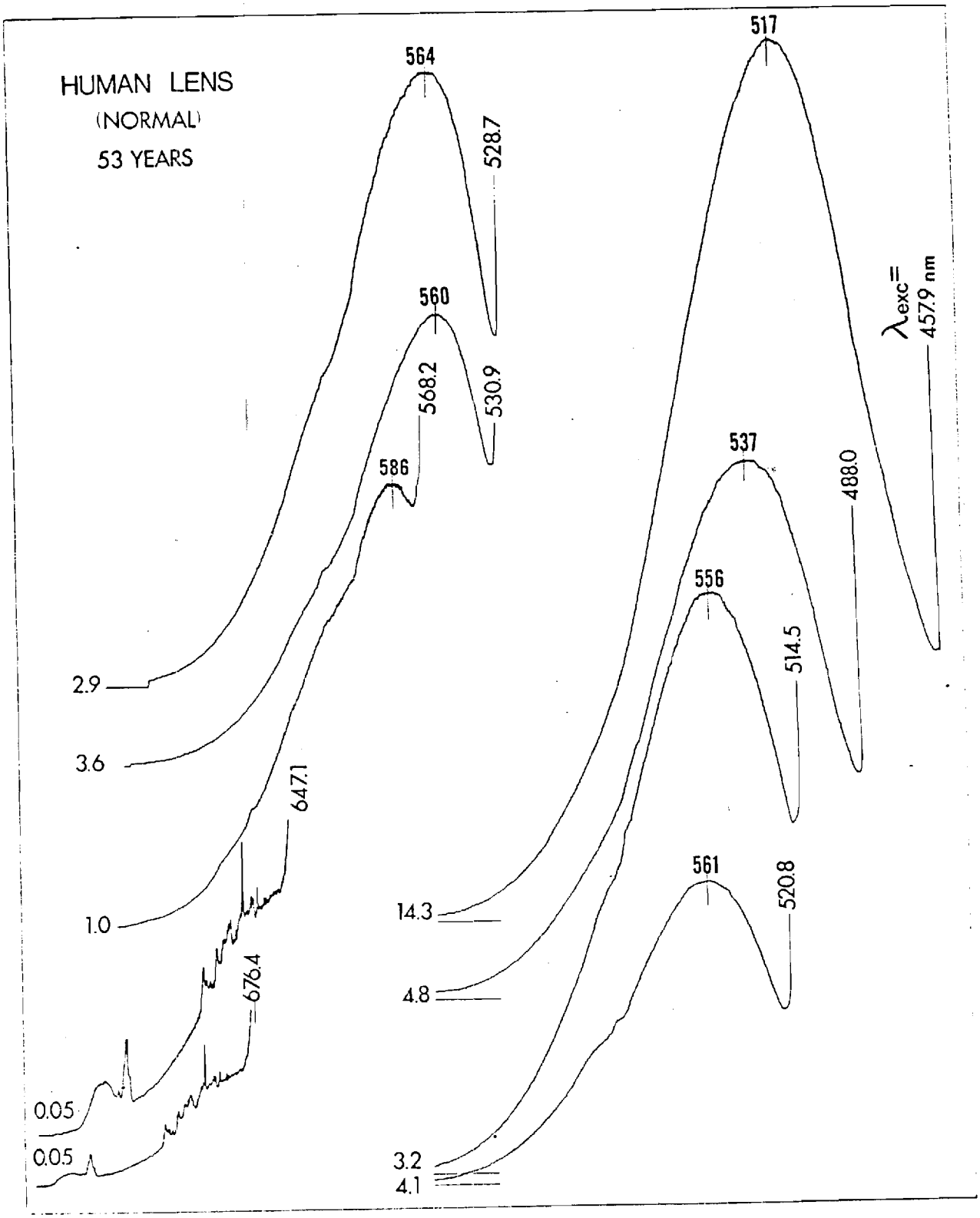
(3) Research Goals in the Coming Year: (a) to develop a multichannel system for UV laser excited fluorescence spectra of human and animal lenses; (b) to measure the accumulation of non-tryptophan fluorescnce (excited at 363.8 nm) in rat and mouse which are grown under dark conditions; (c) to initiate a systematic investigation of isolated lens constituents by a combination of Raman and fluorescence spectroscopy and (d) to measure the rate of Trp deuterium exchange in the nucleus of intact aging lenses.

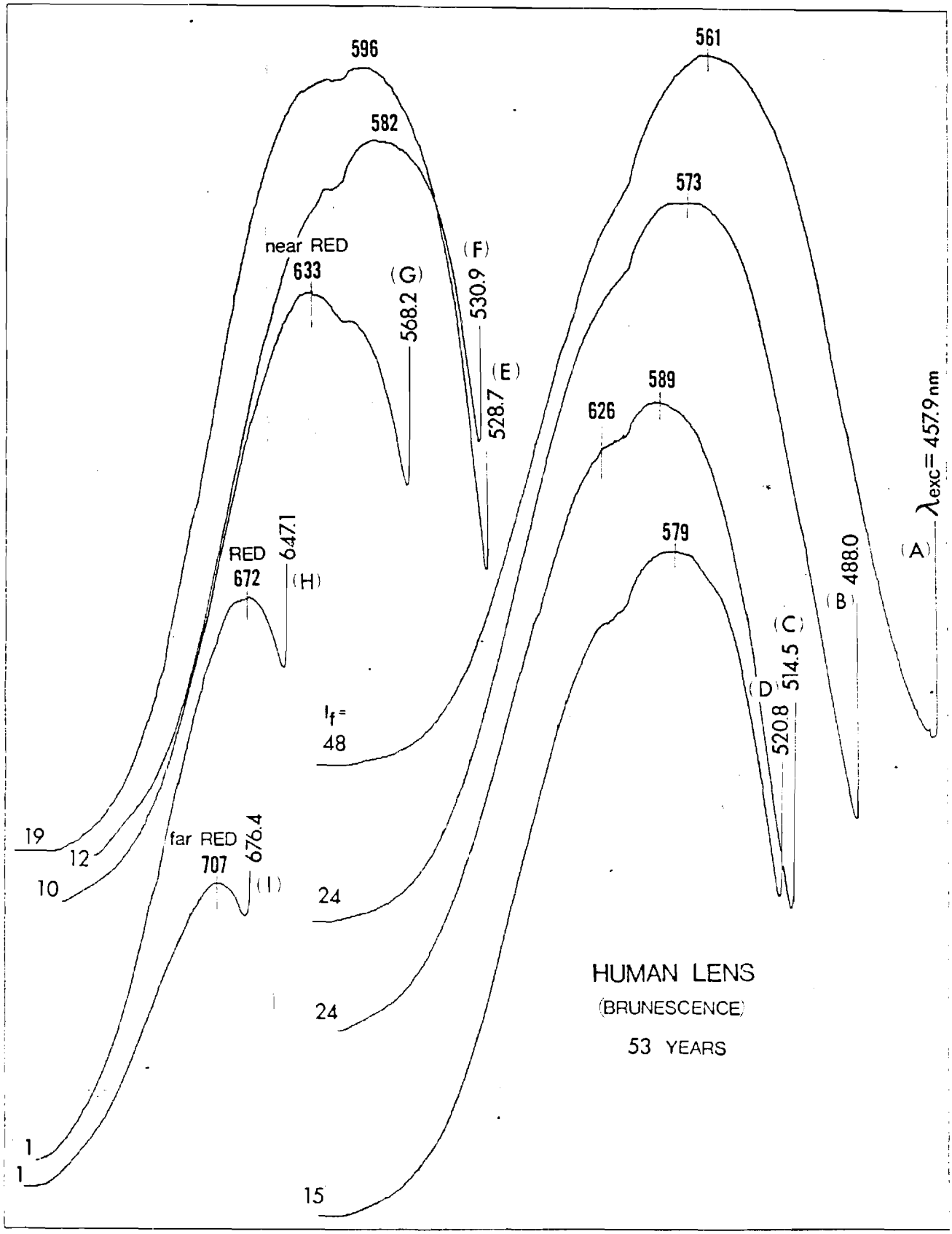
The undersigned agrees to accept responsibility for the scientific and technical conduct of the project and for provision of required progress reports if a grant is awarded as the result of this application.

Dec. 20 1981
Date

Principal Investigator


HUMAN LENS
14 YEARS
489
528
457.9
(B)
488.0
(C)
538
514.5
(D)
(A)
λ_{exc}= 406.7nm
3.6×10^4 cps/mW
4.0×10^3
1.8×10^3
6.0×10^2
Amide A & H_2O
x 3
ν(C-H)
Amide I
Phe
(E)
514.5 = λ critical
(F)
530.9
(G)
568.2
(H)
647.1
10.0 1000.0
12.0 833.3
14.0 714.3
16.0 625.0
18.0 555.6
20.0 500.0
22.0 454.5
24.0 kK 416.7nm


HUMAN LENS
(NORMAL)
53 YEARS
564
528.7
560
568.2
530.9
586
517
537
488.0
556
514.5
561
520.8
λexc= 457.9 nm
2.9
3.6
1.0
0.05
0.05
647.1
676.4
14.3
4.8
3.2
4.1


596
582
561
573
589
626
579
near RED
633
(G)
568.2
(F)
530.9
(E)
528.7
RED
672
647.1
(H)
far RED
707
676.4
(I)
(A)
λexc= 457.9 nm
(B)
488.0
(C)
514.5
(D)
520.8
If =
48
24
24
19
12
10
1
1
15
HUMAN LENS
(BRUNESCENCE)
53 YEARS

October 21, 1980

Curriculum Vitae

Name Masayasu Bando Sex Male Age 33 yrs

Birth Date March 27, 1947 Place of Birth Tokyo

Title Instructor, Department of Ophthalmology, School of Medicine, Tokai University, Isehara, Kanagawa 259-11, Japan

Dwelling Place

Takanashi-so 105, Ishida 206-13, Isehara, Kanagawa 259-11, Japan

Education

1965 - 1969 Applied Chemistry, Tokyo Institute of Technology

1969 - 1971 Biochemistry, Master Course, Tokyo Institute of Technology

Degree Doctor of Medical Science (This degree was given from Juntendo University School of Medicine on September 9, 1980.)

Research and/or Professional Experience

1971 - 1980 Associate in Ophthalmic Research, Laboratory of Biochemistry, Department of Ophthalmology, Juntendo University School of Medicine, Bunkyo-ku, Tokyo 113, Japan

1980 - date Instructor of Ophthalmic Biochemistry, Department of Ophthalmology, School of Medicine, Isehara, Kanagawa 259-11, Japan

Publications

1. I. Kotoku, A. Matsushima, M. Bando and Y. Inada (1970). Tyrosine and tryptophan residues and amino groups in thrombin related to enzymic activities. Biochim. Biophys. Acta, 214, 490 - 497.

2. Y. Inada, M. Bando, I. Kotoku, A. Matsushima and J. Hirana (1971). Enzymic action of thrombin on fibrinogen compared with that of trypsin. Biochim. Biophys. Acta, 251, 94 - 95.

3. M. Bando, A. Matsushima, J. Hirano and Y. Inada (1972). Thrombin-catalyzed conversion of fibrinogen to fibrin. J. Biochem., 71, 897 -899.

4. K. Satoh, M. Bando and A. Nakajima (1973). Fluorescence in human lens. Exp. Eye Res., 16, 167 - 172.

5. M. Bando (1973). The relationship between coloration and fluorescence in human lens. Acta Soc. Ophthalmol. (Japan), 77, 873 - 876.

6. M. Bando, A. Nakajima and K. Satoh (1975). Coloration of human lens protein. Exp. Eye Res., 20, 489 - 492.

7. M. Bando and M. Tsukada (1975). Adsorption of polycyclic compound to uveal melanin granule. Acta Soc. Ophthalmol. (Japan), 79, 1528 - 1532.

8. M. Bando, A. Nakajima, M. Nakagawa and T. Hiraoka (1976). Measurement of protein distribution in human lens by microspectrophotometry. Exp. Eye Res., 22, 389 - 392.

9. M. Bando, Y. Ishii and A. Nakajima (1976). Changes in blue fluorescence intensity and coloration of human lens protein

with normal lens aging and nuclear cataract. Ophthalmic Res., 8, 456 - 463.

10. K. Satoh and M. Bando (1976). A comparative study of rabbit lens proteins. Documenta Ophthalmologica Proceedings Series Vol. 8, 113 - 120.
11. T. Yamaguchi, M. Bando, A. Nakajima, M. Terai and M. Suzuki (1977). Simultaneous determination of trace elements in human eye tissues by neutron activation analysis. Metabolic Ophthalmol., 1, 149 - 152.
12. T. Yamaguchi, M. Bando, A. Kanai and A. Nakajima (1978). Phospholipid in human cornea of the granular dystrophy. Acta Soc. Ophthalmol. (Japan), 82, 393 - 397.
13. M. Bando, A. Nakajima and K. Satoh (1978). Association of sugar with human lens protein. Interdiscipl. Topics Geront., 13, 231 - 238.
14. T. Yamaguchi, M. Bando, A. Kanai and A. Nakajima (1979). Phospholipid in human cornea of the granular dystrophy. Metab. Pediat. Ophthal., 3, 195 - 201.
15. T. Yamaguchi, M. Bando, A. Nakajima, M. Terai and M. Suzuki-Yasumoto (1980). An application of neutron activation analysis to biological materials. IV. Approach to simultaneous determination of trace elements in human eye tissues with non-destructive neutron activation analysis. J. Radioanal. Chem., 57, 169 - 183.
16. S. Rasmidatta, A. Fukushima and M. Bando (1980). Ipratropium bromide - effects on the eye. Acta Soc. Ophthalmol.

(Japan), in press.

17. S. Kittiponghansa and M. Bando (1980). Evaluation of continuous wear of high water content soft lens in albino rabbit by analysis of protein and lactic acid in the tear. J. Jpn. C.L. Soc., in press.

18. M. Bando, A. Nakajima and K. Satoh (1980). Spectrophotometric estimation of 3-OH L-kynurenine O-β-glucoside in human lens. J. Biochem., in press.

19. M. Bando, I. Mikuni and H. Obazawa (1980). Reactivity of 8-methoxypsoralen to lens protein. Photomedicine and Photobiology (Japan), Vol. 2, in press.

Prepared for the Science Information Exchange.
Not for publication or publication reference.

U. S. Department of
HEALTH, EDUCATION, AND WELFARE
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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

TITLE OF PROJECT

Comparative Raman Studies of Human and Animal Lenses

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Nai-Teng Yu (Principal Investigator) Professor of Chemistry
J. F. R. Kuck, Jr. Research Collaborator, Dept. of Ophthal. Emory Univ.
M. Bando, Research Associate
C. Pace, Research Associate

NAME AND ADDRESS OF APPLICANT INSTITUTION.

Georgia Institute of Technology, 225 North Ave., Atlanta, Ga. 30332

SUMMARY OF PROPOSED WORK—*(200 words or less) — Omit Confidential data.)*
In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

1. Fluorescence spectra of pigmented human(age 23-97, normal and brunescent) and animal(chipmunk, 1 yr.) lenses have been obtained with laser excitation at 406.7, 413.1, 454.5, 457.9, 465.8, 476.5, 488.0, 496.5, 501.7, 514.5, 520.8, 530.9, 568.2, 600.0, 610.0, 620.0, 630.0, 647.1,and 676.4 nm. All the lenses examined exhibited strong and similar fluorescence when the excitation wavelengths are shorter than 460 nm. However, with excitation at 568.2 nm or longer the yellow pigments in chipmunk lens show dramatically different emission properties from those in the older and brunescent human lenses.

2. The sulfhydryl concentration in the central nucleus of rat and mouse lenses falls precipitiously with age. However, in the lenses of man and water buffalo the sulfhydryl decreases at a much slower rate with age. This difference between the two groups appears to be correlated with the derivation of albuminoid: in the rodents it is chiefly γ-crystallin which gives rise to albuminoid while in human and bovine lenses albuminoid is related to α-crystallin.

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
		2/20/81

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED	BEGINNING DATE	ESTIMATED COMPLETION DATE

PHS-166 (Formerly NIH-1946)
REV. 9-71